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Gene loss in keratinization programs accompanies adaptation of cetacean skin to aquatic lifestyle

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The transition to a fully aquatic lifestyle in cetaceans, baleen and toothed whales, was accompanied by sweeping changes in their body plan, anatomy and physiology. Detailed fossil records of ancestral forms between land-dwelling mammals and modern-day cetaceans enshrine whales in biology textbooks as an example of macroevolution (1). The advent of mainstream sequencing technologies has allowed the whole genomes of several cetacean species to be assembled and provisionally annotated. This exciting advance enables studying the genetic basis of mammalian adaptation to life in water, including changes to skin. Genomewide profiles of adaptive mutations in cetaceans can facilitate identification of the genetic causes of rare inherited disorders in humans with whale-like skin manifestations: loss of epidermal barrier function and loss of appendages.

Cetacean integument is uniquely suited for an aquatic lifestyle. Their epidermis is smooth and rubber-like on the outside. On the inside, it is exceedingly thick and forms deep, root-like projections into the underlying dermis. This anatomy produces a dramatically increased basal-to-outer surface ratio and results in an expanded basal progenitor compartment, enabling a high epidermal turnover rate. Cetacean skin also has a simplified repertoire of cutaneous appendages: pelage hairs, sweat glands and claws are absent, but few sensory vibrissae form. Interestingly, while in baleen whales vibrissa follicles cycle throughout life, in the majority of dolphins at birth they convert into small, highly innervated sensory pits (2). The general ability of cetaceans to form vibrissae suggests that their lack of body hair is likely caused by the suppression of hair patterning rather than a defect in the hair follicle morphogenesis program *per se*. Preservation of the follicle-forming program in dolphins for the sake of developing sensory pits highlights the overall importance of the tactile perception of skin appendages, a feature that also co-evolved in birds in form of filoplumes, sensory vibrissa-like feathers (3).

Despite its thickness, cetacean epidermis is highly susceptible to dehydration. Indeed, the news commonly covers dramatic rescue attempts of dolphins and whales at high risk of dying once stranded on the beach, with need to be kept constantly wet. A recent study by Strasser *et al.* (4) lends new insight into the genetic basis of what appears to be an epidermal barrier defect in cetaceans. In most land mammals, the epidermis acquires its barrier function through a terminal differentiation program that includes: (i) aggregation of keratin filaments into bundles, (ii) assembly of the cornified envelope and (iii) sealing of the intercellular spaces with lipids (5). Keratin filament bundling in mammalian epidermis is coordinated by filaggrin, an abundant intermediate-filament binding protein. Genetically, the filaggrin precursor is encoded as part of the larger epidermal differentiation complex (EDC), a prominent gene cluster which includes: (a) multiple calcium-binding S100A proteins; (b) cornified envelope precursors, such as involucrin and loricrin; and (c) other intermediate-filament binding proteins, also known as S100 fused-type proteins (SFTPs) (6). However, in cetaceans things appear somewhat different.

By comparing the genomes of five different cetacean species, Strasser *et al.* (4) made the striking observation that all SFTP genes, with the notable exception of filaggrin, become pseudogenized via introduction of premature stop codons, frameshifts, gaps in exonic sequences or a combination of sequence alterations. In many instances, SFTP homologs were not detected via sequence homology, suggesting these genes were deleted during evolution, or perhaps remain hidden in gaps in the current genome assembly. The latter possibility is a common challenge faced during assembly of highly repetitive genomic regions such as the EDC or HOX gene clusters (7). The assembled sequence size of the EDC is significantly larger in humans and cows, whose genome assemblies are more complete, as compared to cetaceans and pigs, whose genomes are assembled only provisionally and contain a large number of ambiguous bases (Fig. 1).

While filaggrin was shown to be present, and tentatively functional, in bottlenose dolphins, killer whales and Chinese river dolphins, it was not detected (either due to being deleted *in vivo*, or not yet mapped *in silico*) in sperm whales and minke whales. Phylogenetically, sperm whales belong to the suborder of toothed whales (*Odontoceti*) together with dolphins, while the minke whale is a baleen whale (suborder of *Mysticeti*). Thus, filaggrin loss in sperm and minke whales would presumably require two independent evolutionary events, one in baleen whales and another in the subset of toothed whales. Studies in mice and humans indicate

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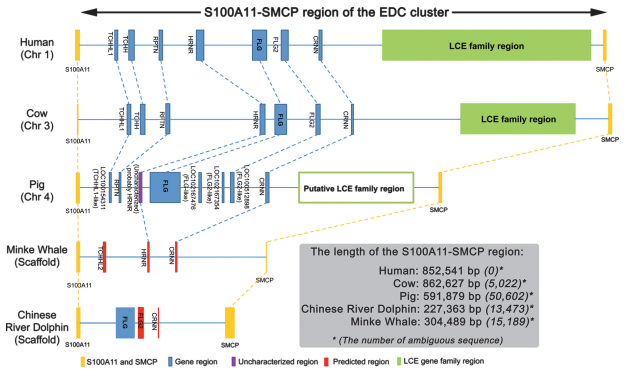

Figure 1. Annotated size of the EDC gene cluster portion in different species. The assembled S100A11-SMCP region of the EDC cluster is shorter in species with provisionally assembled genomes, minke whales, Chinese river dolphins and pigs, compared to genomes of humans and cows. The size of the annotated region inversely correlates with the number of ambiguous, unassembled sequences (grey box). Known genes are marked with blue, predicted genes with red and uncharacterized regions with purple. S100A11: S100 calcium-binding protein A11, SMCP – sperm mitochondria-associated cysteine-rich protein (SMCP is part of EDC, but has unrelated function in sperm motility), TCHH – trichohyalin, TCHHL1 – trichohyalin-like 1, RPTN – repetin, HRNR – hornerin, FLG – filaggrin, FLG2 – filaggrin family member 2, CRNN – cornulin, LCE – late cornified envelope proteins.

that pseudogenization of SFTPs can contribute to the disruption of the epidermal barrier. Prominently, a loss-of-function mutation in filaggrin in 'flaky tail' mice disrupts their epidermal barrier and enhances transepidermal antigen transfer (8). Similarly, filaggrin mutations in humans strongly predispose for the development of atopic dermatitis, which results from the increased transcutaneous antigen penetration and subsequent immune reaction (9). Loss of other SFTPs, such as filaggrin 2, was also shown to contribute to epidermal barrier disruption (10). Future studies comparing sequences of other EDC genes, especially components of the cornified envelope, are likely to provide additional insight into the loss of epidermal barrier function in cetaceans.

Consistent with the findings of Strasser *et al.* (4), two recent studies reveal high levels of gene deletion and pseudogenization in other gene clusters involved in keratinization, α-keratin and keratin-associated protein (KRTAP) clusters. This suggests evolutionary relaxation of selection (i.e. disabling mutations are allowed to accumulate) as a general mechanism driving adaptation of mammalian integument to an extreme ecological niche change (11,12) (see Data S1). In the future, cetacean pseudogene maps can be referenced to facilitate identification of the genetic causes of orphan human diseases manifested by defects in epidermis, sweat glands and hair follicles. Generally, emerging molecular data support the notion that loss of functional significance, that is, that of epidermal barrier function and fur coat in cetaceans, was accompanied by relaxation in evolutionary selection in related terminal differentiation pathways. Importantly, analogous genetic changes occurred during evolution of human skin – compared to chimpanzees, humans have a premature stop codon in the hair keratin gene KRT41P (13), a mutation that likely coincided with a decreased importance for body hair coverage in hominids.

Does the dramatic loss of genes encoding keratinization proteins indicate a general trend for anatomical simplification of ectodermal structures? Not always. Palatal epithelium in baleen whales has evolved into a highly specialized keratinized derivative structure, the baleen, which acts as a massive filter. Baleen is organized into racks of parallel plates. Tongue movements abrade the lingual surface of baleen plates to expose individual hair-like keratinous tubules, which act as sifting units (14). Intriguingly, tensile properties of baleen tubules were shown to be substantial, albeit lower than that of hair. Moreover, baleen in some whales, such as the sei whale, was shown to be highly mineralized (close to 14%), and mineralization significantly contributes to its tensile properties (around 40%) (15).

Taken together, the studies by Strasser *et al.* (4) and others (11,12) identify cetacean skin as an attractive novel model system for examining the effects of gene loss on skin formation and function. Recent advances in induced-pluripotent stem cell (iPSC) technologies (16) may soon enable working with dolphin and whale iPSC-derived skin cells in the laboratory settings using organotypic culture assay for studying epidermal differentiation and barrier function, or 'patch' assay for studying hair formation and hair loss.

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Author contributions

JWO and MVP wrote the paper, OC, YSC and GRM critically revised the paper.

Conflicts of interest

The authors have declared no conflicting interests.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Data S1. Supplementary Text
Figure S1. Vibrissa hairs of Cetaceans.
Supplementary References. S1-S4

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Supporting information

Mammals have two clusters of α-keratin genes, type I (acidic) and type II (basic). Within these, a portion of α-keratins are primarily expressed in hair shafts, hence they are called hair-type keratins (s1, s2). Differentiation of hairs is also highly dependent on the so-called keratin-associated proteins (KRTAPs), diverse structural proteins, whose genes are arranged into five distinct clusters, with one nested inside the type I α-keratin gene cluster (s3). Intriguingly, Nery *et al.* (s1) showed that hair-type keratins are largely lost from type I and type II gene clusters in Bottlenose dolphins and Minke whales via gene deletion and pseudogenization (note that α-keratin gene clusters in Cetaceans are only partially mapped probably due to the provisional state of their genome assembly). In the context of available data, Minke whales show dramatic depletion of cluster I, with only one hair-type keratin, KRT23, remaining intact. In another study, Khan *et al.* (s3) reported a very high rate of pseudogenization (74%) in KRTAP gene clusters in Bottlenose dolphins, compared to other land mammals (averaging 19% pseudogenization). Taken together, this data suggests that although vibrissa follicles in Cetaceans can, in principle, produce keratinized hair filaments (Figure S1), their biomechanical properties likely differ compared to those of terrestrial mammals. Interestingly, some anatomical and physiological features of Cetacean skin can be identified in Hippos (*Hippopotamus amphibious,* order *Artiodactyla*), the closest living semiaquatic relative of dolphins and whales. Similar to Cetaceans, the epidermis of Hippos is thickened and features deep, root-like projections, as well as high transepidermal water loss (s4). Future studies comparing Cetacean and Hippopotamus genomes (once made available) are likely to reveal which changes in the skin differentiation program evolved as exclusive adaptations to fully aquatic *vs.* intermediate, amphibian lifestyles.

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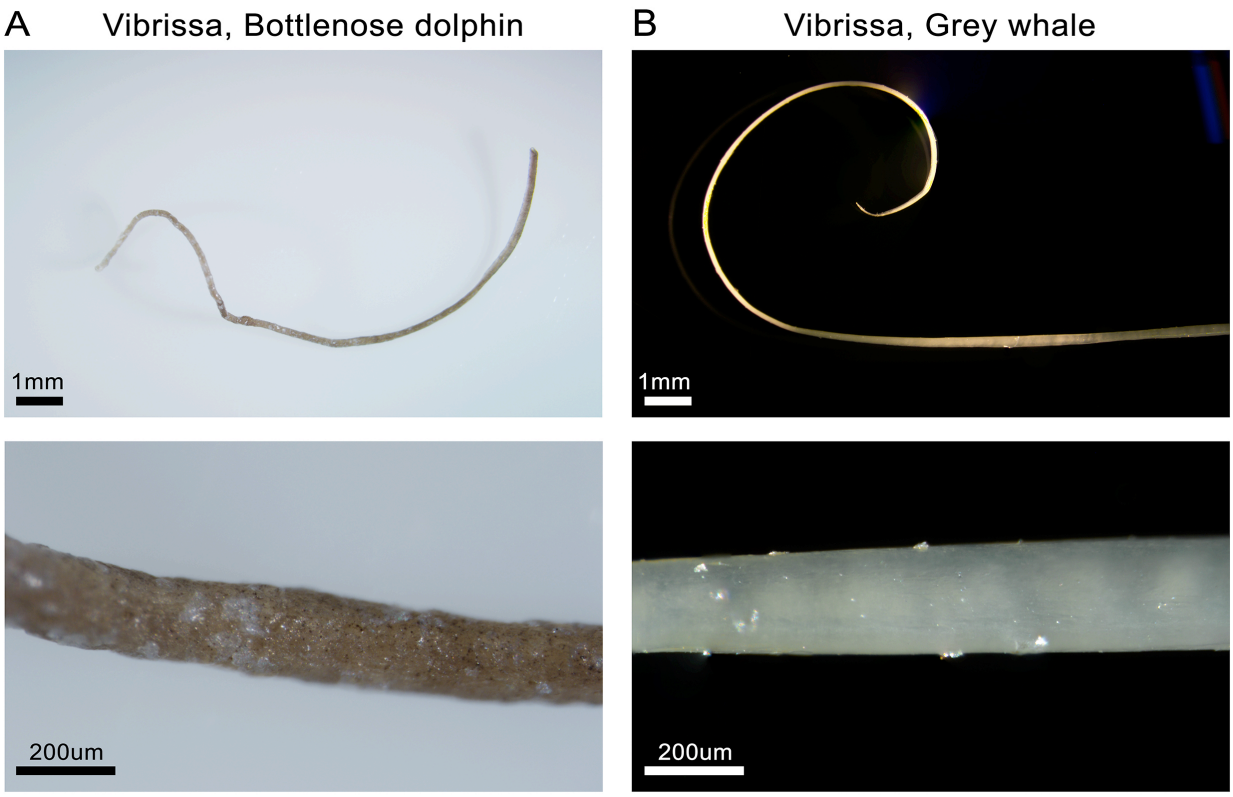

Supplementary figure S1: Vibrissa hairs of Cetaceans. Vibrissa follicles in adult Grey whale **(A)** and newborn Bottlenose dolphin **(B)** are able to produce differentiated hair filaments.